

Interaction between Lymphocytes and Platelets in the Synthesis of Prostacyclin

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Abstract

To test the hypothesis that prostacyclin (PGI₂) is formed via a biochemical interaction between platelets and lymphocytes, we measured eicosanoids by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). A distinct 6-keto-prostaglandin F_{1α} (6KPGF_{1α}) peak was noted when [¹⁴C]arachidonic acid ([¹⁴C]AA) was added to the mixed cell preparations which was increased by pretreating platelets with 1-benzylimidazole (1-BI). Lymphocytes prelabeled with [¹⁴C]AA failed to form 6KPGF_{1α} when stimulated with phytohemagglutinin (PHA) or ionophore A23187. When the prelabeled platelets were suspended together with aspirin-treated lymphocytes and stimulated with ionophore, thrombin, or collagen, a 6KPGF_{1α} peak was detected and enhanced by 1-BI. These results were supported by quantifying the 6KPGF_{1α} content in the HPLC-purified fraction by RIA. Adding prostaglandin H₂ (PGH₂) directly to lymphocytes led to 6KPGF_{1α} production. Platelet aggregation and release were inhibited by lymphocytes in a dose-related manner. We conclude that lymphocytes possess PGI₂ synthase activity which is capable of converting platelet-derived PGH₂ into PGI₂. PGI₂ formed is sufficient to inhibit platelet function.

Introduction

Human lymphocytes are capable of taking up arachidonic acid (AA)¹ and incorporating it into their phospholipids, yet they appear to possess minimal ability to convert that arachidonate into biologically active metabolites (1, 2). For example, Parker et al. detected the production of only small quantities of thromboxane B₂ (TXB₂), 5-hydroxyeicosatetraenoic acid (5-HETE),

and 12-HETE upon treating lymphocytes with phytohemagglutinin (PHA) (1). These metabolites were thought to be due to contamination of the lymphocyte preparation with platelets and neutrophils. Goldyne and Stobo observed that T lymphocytes prelabeled with [¹⁴C]AA failed to synthesize eicosanoids although they did release free [¹⁴C]AA (2). Hence, lymphocytes probably contain little or no cyclooxygenase activity to metabolize AA into active products such as TXA₂ or prostacyclin (PGI₂).

Recent data have shown that the serum content of 6-keto-prostaglandin F_{1α} (6KPGF_{1α}) is significantly higher than that of plasma (3, 4). This difference must be due to PGI₂ synthesis by blood cells stimulated during the coagulation process. However, the identity of the cells responsible for PGI₂ synthesis remains unknown. Since none of the isolated blood cell fractions produces PGI₂ directly, one must consider the possibility that PGI₂ is synthesized via a biochemical interaction between two different classes of cells. The purpose of this study was to test the hypothesis that, despite their lack of cyclooxygenase activity, blood lymphocytes possess PGI₂ synthase activity which can convert platelet-derived (PG) endoperoxides into PGI₂.

Methods

Materials. 1-[¹⁴C]AA (56 mCi/mmol sp act) was obtained from New England Nuclear, Boston, MA. AA was obtained from Nu Chek Prep, Inc. (Elysian, MN). ADP and ionophore A23187 were obtained from Sigma Chemical Co., St. Louis, MO and collagen, from Hormon-Chemie, Munich, Germany. Thrombin was obtained from Parke, Davis & Co., Detroit, MI; 1-benzylimidazole (1-BI), from Aldrich Chemical Co., Milwaukee, WI; and PHA, from Wellcome Diagnostic, Greenville, NC. Tritiated PG standards: PGD₂, PGE₂, PGF_{2α}, 6KPGF_{1α}, TXB₂, 12-hydroxyheptadecatrienoic acid (HHT), and 12-HETE, were purchased from New England Nuclear (Boston, MA).

PGH₂ purification. PGH₂ was prepared using AA and ram seminal vesicle microsomes, and the biosynthetically produced PGH₂ was purified over a gravity flow silicic acid column and a cyanobonded phase HPLC self-packed column using a hexane-isopropanol gradient (5). The purified product is free from other eicosanoids as analyzed by gas chromatography-mass spectrometry. This ultrapurified PGH₂ was active in inducing human platelet aggregation in low micromolar concentrations.

Preparation of lymphocytes and platelets. Both lymphocytes and platelets were isolated from venous blood of healthy human subjects. Lymphocyte suspensions were prepared by the Ficoll-Hypaque separation technique (6). Platelet and monocyte contaminations in the lymphocyte preparations were evaluated by light microscopic and scanning electron microscopic examinations. Scanning electron microscope work was performed by Dr. Bucana at University of Texas Cancer Center, Department of Cell Biology. The lymphocyte preparations were contaminated by a small percentage of platelets ranging from 3 to 15%. Monocyte contamination was not detected by either microscopic examination. In some experiments, lymphocytes were inactivated by boiling at 100°C for 3 min. There was no apparent cell agglutination in the boiled lymphocyte preparation. Washed platelet suspensions were prepared according to the method described by Walsh (7). The final preparation was suspended in Tyrode's buffer containing 5 mM calcium and 0.5 mg/ml human fibrinogen. Only freshly prepared cell suspensions were used. To obtain

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1. **Abbreviations used in this paper:** AA, arachidonic acid; 1-BI, 1-benzylimidazole; HETE, hydroxyeicosatetraenoic acid; HHT, 12-hydroxyheptadecatrienoic acid; 6KPGF_{1α}, 6-keto-prostaglandin F_{1α}; PHA, phytohemagglutinin; PPP, platelet-poor plasma; PRP, platelet-rich plasma; PG, prostaglandin; PGI₂, prostacyclin; TX, thromboxane.

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washed buffy coat preparations, venous blood was drawn into tubes containing 1/10 vol of 3.8% sodium citrate, mixed and centrifuged at 3,000 *g* for 20 min. After removing plasma, the buffy-coat layer was carefully separated and washed three times in Tyrode's buffer and the final preparation was suspended in 2 ml Tyrode's buffer that contained 5 mM calcium.

Analysis of eicosanoids by HPLC. Three types of experiments were performed. In the first experiment [14 C]arachidonate was added to lymphocyte suspensions (10^7 cells/ml) alone or with washed platelet suspensions (3×10^8 cells/ml) in a platelet aggregometer cuvette and incubated at 37°C under constant stirring for 3 min. In the second experiment washed platelets were prelabeled with 2 μ M [14 C]arachidonate at 37°C for 2 h. The cells were washed twice to remove free radiolabeled arachidonate. Washed lymphocytes pretreated with 1 mM aspirin were added to the labeled platelet preparation in an aggregometer cuvette for 1 min followed by addition of an agonist (ionophore A23187, thrombin, and collagen) for 3 min. In the third experiment washed lymphocytes were prelabeled with 2 μ M [14 C]arachidonate at 37°C for 1 h. Cells were washed twice to remove free radiolabeled arachidonate. Washed labeled lymphocytes were placed in an aggregometer cuvette and treated with ionophore or PHA for 3 min. We performed all the incubation experiments for 3 min, because at this time period maximal eicosanoids were produced. The reaction was terminated by acidification to pH 3.0. After the samples were neutralized, they were centrifuged and the supernatants were acidified to pH 4.0. Eicosanoids were extracted by using a Sep-Pak cartridge (Waters Associates, Millipore, Corp., Milford, MA) as previously described (8). Recovery for eicosanoids was ~95%.

The eicosanoids were analyzed by reverse phase HPLC (model Vista 5500 HPLC; Varian Associates, Inc., Palo Alto, CA). The prostanooids were separated using an isocratic solvent system of 35% acetonitrile in acidified water. After initial isocratic period, the solvent composition was changed to 50% acetonitrile in acidified water and ran for 10 min to elute HHT, leukotriene B₄, and HETEs. Next, a slight convex gradient was run from 50 to 75% acetonitrile for 8 min and then to 100% acetonitrile. Fractions were collected and the radioactivity was counted in a β -scintillation counter. Tritiated standards were used to determine the relative retention time of each eicosanoid.

RIA. Eicosanoids were separated by HPLC as described above and the fractions corresponding to 6KPGF_{1 α} were pooled and assayed for 6KPGF_{1 α} by a highly sensitive and specific RIA (9). To ascertain that an optimal amount of 12-HETE was produced at 3 min after addition of an agonist, we measured 12-HETE produced by platelet stimulated with 10 μ M AA at 1, 3, 5, and 30 min by RIA (Seragen, Milford, MA). Our data indicated that the production reached plateau at 3 min. 12-HETE at these time points was 663, 852, 700, and 878 ng/ml, respectively.

Platelet aggregation. Platelet aggregation was measured by the turbidimetric method of Born (10) using a dual-channel aggregometer (Chrono-Log Corp., Havertown, PA). The procedure was modified to provide an appropriate control for reduced light transmission caused by the addition of lymphocyte preparation. For example, to determine the effect of lymphocytes on platelet aggregation, the platelet-rich plasma (PRP) cuvette in the aggregometer contained 0.2 ml of lymphocyte preparations (varying concentrations) and 0.8 ml of washed platelet suspensions (3×10^8 /ml), whereas the platelet-poor plasma (PPP) cuvette contained 0.2 ml of lymphocyte preparation at an identical concentration and 0.8 ml of the Tyrode's buffer used to suspend washed platelets. The mixtures were incubated at 37°C under constant stirring for 1 min before addition of the agonists: collagen (2–15 μ g/ml), thrombin (0.05–0.1 U/ml), AA (10–20 μ M), or buffer control. The extent of change in light transmission was continuously recorded for 5 min. In all of the experiments performed using this modified procedure, the addition of lymphocytes to platelet suspensions without agonists caused minimal changes in light transmission. For the regular platelet aggregation experiments in which lymphocytes were not added, buffer alone was placed in the PPP cuvette. The concentration of each agonist was determined experimentally as the minimal concentration inducing maximal aggregation.

The PGI₂-like activity was assayed by relating the percent inhibition of platelet aggregation of a tested sample to a calibration curve constructed by using varying concentrations of authentic PGI₂ as previously described (11).

Platelet serotonin release. Platelet serotonin release was performed according to the method of Weiss et al. (12) with minor modification. In brief, 3×10^8 platelets/ml PRP was labeled with 2 μ M [14 C]serotonin at room temperature for 30 min and 20 μ M imipramine was then added. 0.45 ml of labeled PRP was incubated with 0.05 ml of lymphocytes or buffer in an aggregometer cuvette at 37°C for 6 min under constant stirring. The stirring bar was removed and the sample was centrifuged at 13,000 *g* for 1 min in an Eppendorf microfuge (Brinkmann Instruments, Inc., Westbury, NY). The radioactivity was determined in a β -scintillation counter.

Results

Fig. 1 shows a representative prostanoid profile analyzed by HPLC. A distinct 6KPGF_{1 α} peak was noted when 10 μ M [14 C]AA was added to a mixture of lymphocyte and platelet preparations. This peak was enhanced by pretreating platelets with a TX synthase inhibitor, 5 mM 1-BI, but was abolished by pretreating platelets with 1 mM aspirin. When [14 C]AA was added to a mixture of boiled lymphocytes and platelets, the 6KPGF_{1 α} peak was undetectable, nor was the peak discernible when [14 C]AA was incubated with lymphocytes alone. Lymphocytes prelabeled with [14 C]AA failed to form any discernible eicosanoid peak including the 6KPGF_{1 α} when stimulated with PHA. Prelabeled

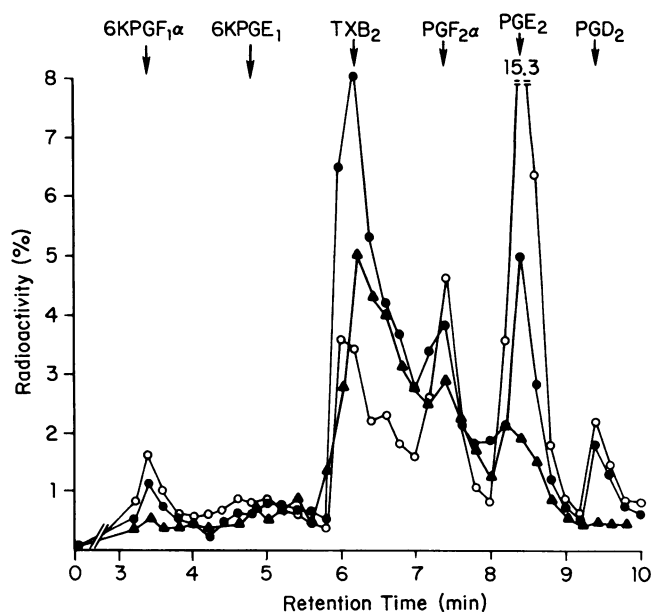


Figure 1. Separation of 6KPGF_{1 α} and other prostanooids by reverse-phase HPLC using the initial isocratic solvent system which excluded HHT, 12-HETE, and AA (see Methods). The arrows refer to the relative retention time of each eicosanoid as determined with tritiated standards. The eicosanoid profile produced by lymphocytes treated with [14 C]AA (\blacktriangle), by a mixture of platelets and lymphocytes incubated with [14 C]AA (\bullet), and when platelets were pretreated with 1-BI before the addition of lymphocytes and [14 C]AA (\circ). Percentages of radioactivity were calculated on the basis of total prostanooids excluding HHT, HETEs, and AA.

Table 1. Eicosanoid Generation by Platelet, Lymphocyte, and Mixed Platelet-Lymphocyte Preparations

Agonists	Eicosanoids						
	6KPGF _{1α}	TXB ₂	PGF _{2α}	PGE ₂	PGD ₂	12-HETE	HHT
	%	%	%	%	%	%	%
Ionophore (10 μM)							
*L	0	7.1	0	1.3	0	13.3	6.3
*P	0	13.7	0	1.9	0	14.0	6.1
*P + 1-BI	0	2.1	2.4	11.5	13.2	13.0	3.0
*P + L	1.2	10.0	0	0	0	13.7	7.9
*P + L + 1-BI	2.0	2.5	3.5	5.9	2.7	7.8	2.0
Thrombin (0.5 U/ml)							
*P	0	8.3	0	1.8	1.2	12.8	5.9
*P + 1-BI	0	2.7	3.3	4.7	5.2	7.5	1.6
*P + L	1.0	9.3	0	1.3	1.2	10.3	7.4
*P + L + 1-BI	1.5	2.4	4.2	6.5	5.0	8.0	3.1
Collagen (10 μg/ml)							
*P	0	4.3	0	2.1	1.6	3.2	2.6
*P + 1-BI	0	1.8	1.9	2.1	2.3	4.9	3.0
*P + L	1.3	4.6	0	1.4	0	3.2	2.6
*P + L + 1-BI	2.0	4.0	4.2	6.1	4.2	4.5	2.6

Experimental details are described in the Methods. After the incubation of cell preparations with each agonist, reaction was terminated and eicosanoids were extracted and analyzed by reverse-phase HPLC. Percentage of each eicosanoid refers to the percentage at the peak point. "0" denotes no discernible peak. *L and *P denote lymphocytes and platelets prelabeled with [¹⁴C]AA, respectively. L denotes aspirin-treated unlabeled lymphocytes.

lymphocytes also did not generate 6KPGF_{1α} when treated with ionophore, although TXB₂, HHT, and 12-HETE peaks were apparent (Table I). When the prelabeled platelets were suspended together with aspirin-treated lymphocyte preparations and stimulated with ionophore, thrombin, and collagen, 6KPGF_{1α} peak was detected and was enhanced by treating platelets with 1-BI (Table I). PGE₂, PGF_{2α}, and PGD₂ peaks were also increased by treating the cells with the TX synthase inhibitor. The 12-HETE peak was discernible in all the cell preparations, including ionophore-treated lymphocytes prelabeled with [¹⁴C]AA. The eicosanoid profile of ionophore-treated lymphocytes was similar to that of ionophore-treated platelets, which was consistent with the view that the eicosanoids released in the stimulated lymphocyte preparation were contributed primarily by the contaminating platelets.

In separate experiments, HPLC fractions corresponding to the 6KPGF_{1α} peak were collected and the 6KPGF_{1α} content was measured by RIA. Results are shown in Fig. 2. Lymphocyte preparations (10⁷ cells/ml) produced 0.18 ng of 6KPGF_{1α} (mean of three experiments) when incubated with 10 μM AA for 3 min at 37°C (Fig. 2 A). Lymphocytes incubated with platelets in the absence of exogenous AA did not produce measurable quantities of 6KPGF_{1α}, yet in the presence of 10 μM AA, lymphocyte and platelet mixtures produced an average of 1.39 ng of 6KPGF_{1α} (almost an eightfold increase over lymphocytes alone) (Fig. 2 B). 6KPGF_{1α} production was further enhanced by pretreating platelets with 5 mM 1-BI (Fig. 2 C). These results suggest that lymphocytes do not metabolize AA directly, but that they possess sufficient PGI₂ synthase activity to synthesize PGI₂ from PGH₂. To test this hypothesis, 5 μM ultrapurified PGH₂ was incubated

with washed lymphocytes. The supernatant was applied to HPLC, and 6KPGF_{1α} content in the HPLC fractions was measured by RIA. Significant quantities of 6KPGF_{1α} were synthesized (Fig. 2 D). PGH₂ incubated in buffer alone under similar

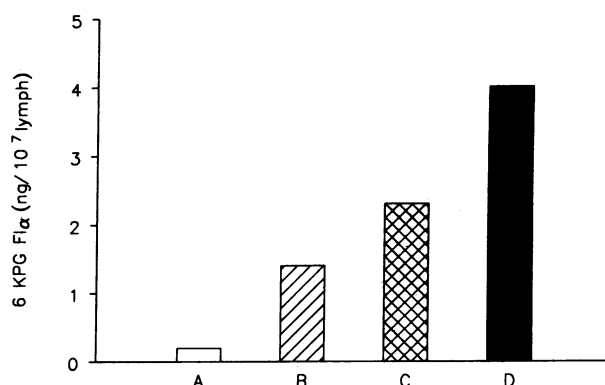


Figure 2. Production of 6KPGF_{1α} by (A) lymphocyte preparations treated with 10 μM AA, (B) mixed lymphocyte and platelet preparations treated with 10 μM AA in the absence of 1-BI, (C) mixed lymphocyte and platelet preparations in the presence of 1-BI, and (D) lymphocyte preparations treated with 5 μM ultrapurified PGH₂. In each experiment, after treatment with AA, the supernatant was collected and the prostanooids were extracted and separated by reverse-phase HPLC. The 6KPGF_{1α} fractions were pooled and their 6KPGF_{1α} content was measured by RIA. The lymphocyte concentration in each experiment was 10⁷/ml and the platelet concentration was 3 × 10⁶/ml. The results represent mean values of two to five experiments.

experimental conditions was not spontaneously hydrolyzed to 6KPGF_{1α}, although it did convert to PGE₂ and PGF_{2α}. The effect of ionophore on PGI₂ synthesis by the mixed cell preparations was evaluated by HPLC separation followed by RIA measurement. Washed lymphocytes were incubated with platelets and then 10 μM ionophore was added. The 6KPGF_{1α} content was comparable to that produced by the cell mixture treated with AA (mean of two experiments was 1.21 ng). Synthesis of 6KPGF_{1α} by washed buffy-coat cells in response to platelet stimuli such as thrombin, collagen, and AA was then determined. Washed buffy-coat cells prepared from 4.5 ml of citrated venous blood were incubated with 10 μM AA, 10 μg/ml collagen, or 0.1 U/ml thrombin at 37°C for 3 min. The reaction was terminated by rapid centrifugation, the supernatant was collected, and its 6KPGF_{1α} concentration was measured by RIA. Mean 6KPGF_{1α} values from the AA, thrombin, and collagen-stimulated preparations were 3.42, 1.42, and 1.68 ng, respectively, whereas the vehicle-treated controls had no measurable 6KPGF_{1α}.

To evaluate the physiologic significance of these observations, we compared the platelet reactivity with three physiologic agonists in the presence and absence of lymphocytes (Fig. 3). Addition of lymphocytes alone to platelets without accompanied agonists did not alter light transmission. Neither did treatment of lymphocytes with the agonists cause lymphocyte aggregation or alter light transmission. By contrast, when lymphocytes were mixed with platelet suspensions and an agonist (AA, thrombin, or collagen) was added, platelet aggregation and [¹⁴C]serotonin release were inhibited (Fig. 3). The degree of inhibition was related to the lymphocyte concentrations added to the platelet suspensions (Fig. 4). To determine whether the inhibiting effect of lymphocytes was primarily due to PGI₂ generation, we measured the 6KPGF_{1α} content in the aggregometer supernatant by bioassay and RIA. Platelets (3×10^8 /ml) and lymphocytes (1×10^7 /ml) were co-incubated by 37°C in an aggregometer cuvette for 1 min followed by addition of 10 μg/ml collagen. Platelet aggregation was continuously recorded. At the end of 5 min, the cuvette was removed from the aggregometer, the stirring bar was removed, and the sample was rapidly spun at 13,000 *g* to remove the cells. The 6KPGF_{1α} content in the supernatant was determined by RIA. The antiaggregatory activity of PGI₂ in the sample was determined by relating the percent inhibition of platelet aggregation to a calibration curve established in each experiment. The mean PGI₂ antiaggregating activity from three experiments was 1.56 ng/ml, whereas the 6KPGF_{1α} content measured by RIA was 1.48 ng/ml, hence, similar values were obtained by two very different assay methods.

Discussion

Our data support the hypothesis that PGI₂ is produced through the interaction of blood platelets and lymphocytes. Three separate assays (i.e., HPLC, RIA, and bioassay) have corroborated the conclusion that these two cells can cooperate to produce a quantity of PGI₂ sufficient to inhibit platelet aggregation and release reaction. That the active component is indeed PGI₂ is supported by two lines of evidence. First was the support by the RIA data on the HPLC-purified 6KPGF_{1α}, which in other studies in our laboratory has correlated well with values obtained by

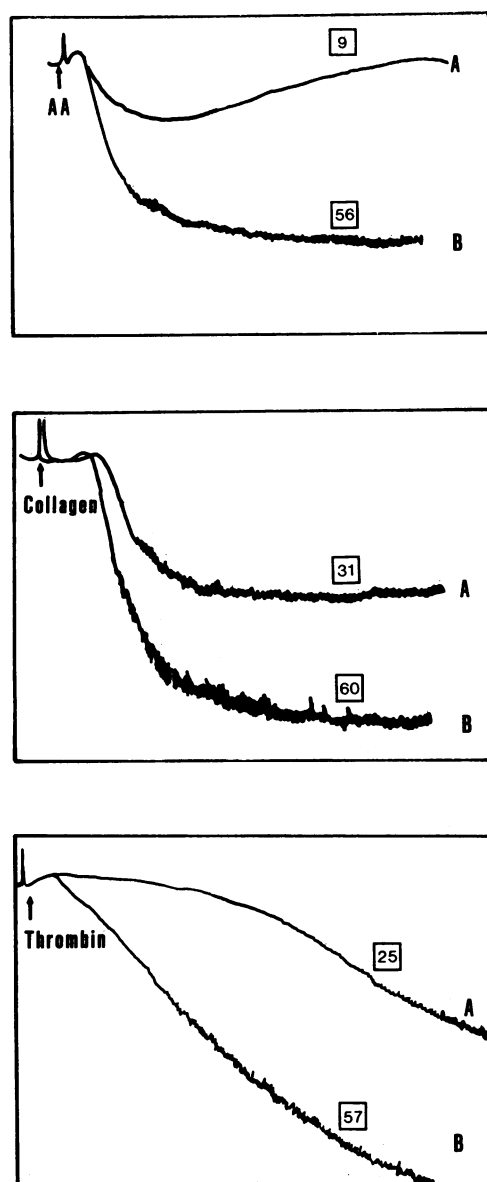


Figure 3. Platelet aggregation induced by 10 μM AA, 10 μg/ml collagen, and 0.1 U/ml thrombin in the presence (tracing A) and absence (tracing B) of lymphocytes. The results are representative of 10 reproducible experiments. The platelet concentration in the washed platelet suspension was 3×10^8 /ml, whereas the lymphocyte concentration was 1×10^7 /ml. Platelet-release reaction measured by [¹⁴C]serotonin release was performed separately under similar experimental conditions. The number indicated in each box at the top of the aggregation tracing denotes the mean percentage of serotonin release of two experiments done in duplicate.

gas chromatography-mass spectrometry (13). The second line of evidence is afforded by the close approximation of the 6KPGF_{1α} values obtained by the HPLC-RIA and the bioassay.

The notion that lymphocyte PGI₂ production depends on platelet-generated PGH₂ is consistent with several of our observations. For instance, PGI₂ production by the platelet-lymphocyte mixture requires the initial activation of platelets. Pretreatment of platelets with a cyclooxygenase inhibitor abolishes lymphocyte

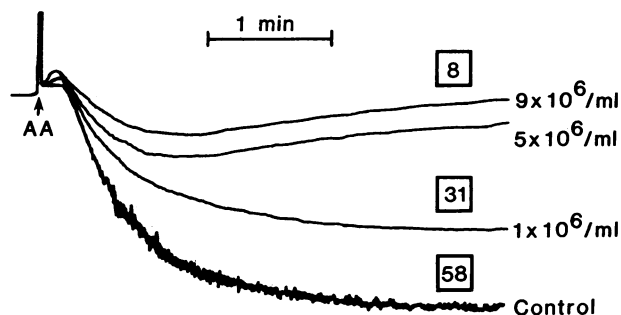


Figure 4. Inhibition of platelet aggregation by various concentrations of lymphocytes. The platelet concentration was fixed at 3×10^8 /ml. The lymphocyte concentrations are indicated with each tracing. To correct the influence of lymphocyte mass on light transmission, the PPP cuvette contained the same lymphocyte concentration as the PRP cuvette. Platelet aggregation was induced with $10 \mu\text{M}$ AA. Serotonin release experiments were performed separately under similar conditions. An identical AA concentration was employed to induce release reaction. The number shown in each box denotes mean percentage of [^{14}C]serotonin release of two experiments done in duplicate.

phocyte PGI_2 production, whereas blocking platelet TX synthase activity with a specific inhibitor enhances it. PGI_2 is formed when aspirin-treated lymphocytes are incubated with radiolabeled platelets and then stimulated with calcium ionophore, thrombin, or collagen, whereas lymphocytes alone fail to produce PGI_2 despite stimulation with PHA and ionophore. Direct evidence was provided in this study by demonstrating a significant production of $6\text{KPGF}_{1\alpha}$ by washed lymphocytes treated with a highly purified PGH_2 preparation. Availability of this pure PGH_2 preparation permits us to document unequivocally that lymphocytes possess sufficient PGI_2 synthase enzyme to metabolize PGH_2 into biologically active PGI_2 .

We have compared the major metabolites in lymphocyte, platelet, and lymphocyte-platelet preparations stimulated with the calcium ionophore. Except for the presence of $6\text{KPGF}_{1\alpha}$, which is formed in the platelet-lymphocyte mixture only, major metabolites generated in these three cell preparations are similar. These data, particularly the approximation of 12-HETE production in all three preparations, indicate that these metabolites are generated by platelets. Measurement of 12-HETE is useful for assessing monocyte contamination because blood monocytes do not synthesize 12-HETE. A significant monocyte contamination should lower the 12-HETE content in the lymphocyte preparation. Based on the eicosanoid profile and our microscopic data, we conclude that the eicosanoids generated by lymphocyte preparation are derived from platelet and not from monocyte contaminants. The contaminated platelet concentration (10^6 /ml), however, is too low to provide lymphocytes with sufficient PGH_2 to synthesize a detectable quantity of PGI_2 . The eicosanoid data shown in Table I also indicate that both calcium ionophore and thrombin produce a similar eicosanoid profile when added to the lymphocyte-platelet mixture, whereas collagen is a weaker stimulator of AA metabolism. Blocking of platelet TX synthase in all these preparations leads to formation of a discernible quantity of PGD_2 and PGE_2 . These compounds may further enhance the antiplatelet aggregating effect of PGI_2 produced in the same mixture.

Treatment of platelets with physiologic agonists in the presence of lymphocytes triggers the production of sufficient PGI_2 to inhibit platelet aggregation. Changes in platelet aggregation tracings are not merely due to the masses of lymphocytes, which may impede the light transmission, because (a) the inhibiting effect was evident when a separate platelet function marker (i.e., [^{14}C]serotonin release) was used, (b) the inhibiting effect was related to the concentration of the agonist added without changing lymphocyte and platelet concentrations and therefore should not be due to an artificial effect of lymphocyte masses, (c) the antiplatelet effect correlated well with the quantity of PGI_2 produced as measured by both the bioassay and the RIA, and (d) each experiment had a carefully standardized internal blank control. Inhibition of platelet function depends on the lymphocyte concentration. In fact, even 10^6 lymphocytes per millimeter of platelet preparation that contained 3×10^8 platelets exhibited appreciable platelet inhibition.

Our observations resolve the mystery of why serum $6\text{KPGF}_{1\alpha}$ levels are higher than the plasma levels. Both the buffy-coat cells and the platelet-lymphocyte mixture experiments indicate that during blood coagulation, generation of thrombin leads to platelet activation and initiation of AA metabolism. Platelet-derived PGH_2 is released and utilized by lymphocytes to form PGI_2 . It is unclear whether this process requires direct cell-to-cell contact. Nevertheless, it may represent an important mechanism whereby PGI_2 can be effectively produced without relying on a single cell source. Platelets appear to function as a donor of PGH_2 for several other cells to synthesize PGI_2 . For example, we have recently shown that PGI_2 is produced by subendothelial smooth muscle cells through platelet donation of PGH_2 (14). Platelets are also donors of PGH_2 for cultured endothelial cells to synthesize PGI_2 (15–17). Although the individual quantity of PGI_2 produced by each cell-cell interaction is small, summation of the total PGI_2 produced will have a powerful biological activity. This may represent a major mechanism whereby PGI_2 acts as an effective autocrine in modulating hemostasis, inflammation, and immune responses.

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